REMARKS

This is in response to the Office Action dated November 30, 2005 for which response was filed May 22, 2006 in the form of an Amendment and a Notice of Appeal, and for which response is due July 22, 2006. Such response due is being filed in the form of a Request for Continuing Examination with this present amendment. The Advisory Action of June 28, 2006 is acknowledged.

It should be noted that the cover page of the Advisory Action says that the previous amendment does not place the application in better form for appeal and was not entered. Also note that page 2 of the Office Action states "The response to the final action filed on May 22 under 37 CFR 1.116 has been entered."

It is respectfully submitted that the amendment should have been entered because it did result in the requisite clarity and patentability over the prior art, placing the application in condition for allowance, as admitted by the Examiner's reasons for rejection.

Since the Office Action Advisory of June 28, 2006 states that the amendment dated May 22, 2006 has not been entered, the present amendments to the claims are relative to the claims in the case as of the earlier amendment dated September 7, 2005.

As a result of this present response, claims 34-37, 45, 46, 48, 49, 54-57, 65 and 66 are in the case. Claims 38-44, 50-53, 58 and 59 are withdrawn. Claims 1-33, 47 and 60-64 are cancelled. It is respectfully submitted that this present amendment places the case in condition for allowance and entry of same is respectfully requested.

Claim Objection

There is an objection to claims 54-57 with regard to depending on claim 1. Appropriate correction has been made in claims 54-57 which now depend directly or indirectly on claim 34.

Sequence Requirements

Appropriate reference to sequence information has been made by this present amendment to the specification description of figures on pages 20 and 21, and with respect to the appropriate designation at Page 51, Table 9 and line 34, all of which are amended herewith, as further described below.

Re. Amendments to the Sequence Listing. The Sequence Listing has now been amended: to include from page 51, Table 9, the sequence of downstream cloning primer ORF2Rb as new SEQ ID NO:19; and to include from page 51, line 34, the sequence of the multiple cloning site of plasmid $pGEX_{20}$ as new SEQ ID NO:20. Support for these amendments is found at page 51 of the Specification as filed, at line 9 (i.e. fifth row of Table 9) and at line 34, respectively. The Sequence Listing also has now been amended to correct a typographical error in the "other information" line <223> for SEQ ID NO:3, by changing "ORF2Rb" to "ORF2Rc." Correction of this informality in the listing for SEQ ID NO:3 serves to clearly distinguish that sequence from ORF2Rb of the newly listed SEQ ID NO:19. No change has been made to the sequence set forth in SEQ ID NO:3.

Statement of Identical Computer Readable Form and No New Matter. Applicants hereby state that the amendments to the Sequence Listing, made in accordance with 37 CFR 1.825(a) and included in the enclosed substitute sheets of the Sequence Listing, are supported in the Application as filed, and introduce no new matter, all sequences having already been set forth in the Specification as filed. Applicants further state that

the content of the enclosed substitute computer readable form is identical to that presented on the enclosed substitute sheets.

Re. Amendments to the Brief Description of the Drawings.

The Brief Description of the Drawings has now been amended to include with the description of each sequence presented in Figures 2A to 2D, 3, 4, and 5, on pages 20 and 21, a recitation of its corresponding SEQ ID NO.

Re. Amendments to the Detailed Description. The Detailed Description has now been amended to include with each sequence presented in the text or in a table, a recitation of its corresponding SEQ ID NO.

Each of the paragraphs at page 26, lines 26-27, and at page 50, lines 16-22, has now been amended to correct a typographical error by changing "ORF2Rb" to "ORF2Rc." Table 9 at page 51, which sets forth the sequences of the PCR and cloning primers described in the Application, has now been amended to insert a row (new row 6) for the ORF2Rc cloning primer sequence recited in SEQ ID NO:3. This row also recites that ORF2Rc comprises the nucleotide sequence that is complementary to positions 6943-6968 of the HEV genome. Further support for these amendments is provided as follows.

The designation of positions 6943-6968 of the HEV genome to specify the sequence to which ORF2Rc (SEQ ID NO:3) is complementary, and to which it hybridizes for priming, is merely further structural information for this already-provided sequence, which structural information is readily obtained by visual inspection of the HEV genome sequence already cited in the present Application: DDBJ (the DNA Data Bank of Japan) accession number D11092 (see, e.g., the footnote to Table 9, at page 52 of the Specification).

Moreover, starting position 6943 for the ORF2Rc genomic sequence complement is also readily discernible by comparison with the sequence of ORF2Rb shown in original Table 9, at page 52 hereof. Original Table 9 states that ORF2Rb contains the sequence complement of HEV genome positions 6932-6956. The following sequence alignment of these two downstream primers shows, in the ORF2Rb sequence, the genomic starting position 6943 for ORF2Rc; the added (non-genomic) EcoRI restriction enzyme site of ORF2Rb is shown in bold italics, the genomic portion of ORF2Rb ending at position 6956, as stated in original Table 9.

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                                        6956          6943         6932
                                         |             |            |
ORF2Rb (SID:19)                  ggc***gaattc***ggggggctaaaacagcaaccgcgga
                                         ||||||||||||||
ORF2Rc (SID:3)      ggcgaatccctagcgcggagggggggctaaaaca
                           |                      |
                         6968                   6943
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In addition, that the ORF2Rc genomic complement comprises position 6968 is also clear for the following reasons. SEQ ID NO:3 is described at page 50 as being useful for cloning the entire coding sequence of the novel pE2 peptide. Comparison of the entire pE2 coding sequence (SEQ ID NO:1) with the HEV genomic DNA (DDBJ D11092) shows that the "t-a-g" stop codon of the pE2 coding sequence (bases 640-642 of SEQ ID NO:1) is located at genome position 6966-6968. Further, comparison of these with the sequences of ORF2Rb (in original Table 9) and ORF2Rc (SEQ ID NO:3) shows that, of these two downstream primers, only ORF2Rc encompasses this "t-a-g" stop codon: in the ORF2Rc sequence above, the underlined "c-t-a" (reversed) complement of the "t-a-g" codon is evident, terminating at genome position 6968. As a result, one of ordinary skill in the art would readily recognize that SEQ ID NO:3 (ORF2Rc) would be useful for cloning the entire E2 coding sequence, whereas ORF2Rb (in original Table 9) would be capable of only partial cloning. Thus, in those

occurrences in which "ORF2Rb" is recited in reference to SEQ ID NO:3 or to that downstream primer that is useful for cloning the entire pE2 coding sequence, it is clearly an obvious error for a sequence of a different name (ORF2Rc). Consequently, the Application as filed provides support for the above-described amendments to Table 9 and the various sequence references amended herein.

In light of these remarks and amendments, it is respectfully submitted that Applicant has fully complied with all of the requirements, and at least as to the sequence listing, has fully complied with this requirement on more than one occasion including specifically documenting in the September response to the Examiner the print-out from PTO PAIRS showing that as of January 5, 2005 "CRF is good technically/entered into database."

REJECTION UNDER 35 U.S.C. § 102/103

Claims 34-37, 45, 47, 54-57 and 64-66 are still rejected under 35 U.S.C. 102(b) on the same ground as stated in the previous Office Action as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over by Reyes et al. (US. Patent No. 5,8968,239A).

REJECTION UNDER 35 U.S.C. § 102

Claims 34-37, 45, and 47-49 are still rejected under 35 U.S.C. 102(b) on the same ground as stated in the previous Office Action, as being anticipated by Reyes et al. (B) (US Patent No. 5,741,490A) or Reyes et al. (C) (US Patent No. 5,770,689).

Claims 34. 37, 64 are still rejected under 35 U.S.C. 102(b) on the same ground as stated in the previous Office Action as being anticipated by Khudyakov et al. (Virol. 1994, pp. 390-393).

Claims 34-37, 45-49, 54-57 and 64-66 are still rejected under 35 U.S.C. 102(e) on the same ground as stated in the previous Office Action as being anticipated by Li and al. (US Patent No. 6,514,690 B1).

RESPONSE TO ALL REJECTIONS UNDER 35 U.S.C. § 102/103

All of the aforesaid rejections are respectfully traversed for the reasons given in the previous responses and for the added reasons below.

The Office Action makes the unsupported assumption that if linear epitopes are closely similar (since the two types of proteins share substantial amino acid sequence homology), it necessarily follows that a monomer and homodimer of each is possible. For the reasons given below, this key assumption in the Office Action is not true. Further, this incorrect assumption that forms the basis of the Office Action is understood to be incorrect based on the literature and the teachings of the present specification as set forth below. The cited references and the present specification, when properly understood, demonstrate that the conventional thinking was that homodimers of the present invention do not form and are not used as described for the first time by the present inventors. Accordingly, a declaration of expert on this point is not provided, but would be provided if requested to enhance the understanding. In addition, expert and inventor, if necessary, the inventor of the present application, Professor Ng Mun Hon, would be more than happy to have a phone communication with the Examiner and preferably her supervisor so as to facilitate examination.

It is well known in the art that a protein molecule is a flexible amino acid polymer. In solution, it folds upon itself according to thermodynamic principles and assumes certain 3D structural form. In rare instances, protein molecules may become attached to one another to form homodimer or higher oligomers.

It is also well recognized that antibodies produced against a given protein include two classes of epitopes, namely, those that recognize parts of its amino acid sequence, referred to as linear epitopes, and features of its 3D structural form, referred to as conformational determinants or conformational epitopes.

The unique E2 homodimer and associated immunoreactivity of the invention is further illustrated as in Attachment A hereto "E2 homodimer and associated immunoreactivity".

As shown in the figure of Attachment A, the invention is arrived at on the basis of the unexpected property of E2, a recombinant protein of major structural HEV structural protein encoded by ORF2 of the HEV genome, that is unique and unknown in the art. It is unique and unexpected that the recombinant protein has a strong propensity to interact with one another to form homodimer (see, above Figure).

The unexpected property of E2 to form homodimer is a rare property among known proteins. In other words, even if the monomer of a protein is disclosed, there is no teaching or suggestion that said protein will form homodimer. Thus, the invention for the first time teaches the unexpected formation of the homodimer.

The newly amended independent claims 34 and 66 are based on identification of the 42KD homodimer by SDS-PAGE. The present specification shows, by this method, that the homodimer is dissociated into 23KD monomer, after the protein sample was subject to heat treatment beforehand.

Immunoreactivity of the homodimer was shown by Western blotting probed with anti-sera from HEV infected humans or infected primates. The monomeric form share the same linear epitopes with homodimer and it additionally presents conformational determinant presented by its 3D structure.

However, there is a vast difference between the immunogenizing of the monomer and the homodimer. Comparing the homodimer and the monomer under the same condition, western blotting show that monomer is either not reactive or weakly so. By comparing Western blotting analysis of the unheated and heated samples, it is shown that the unexpected immunogenicity of homodimer is predominantly attributed to conformational determinants presented by 3D structure of the homodimer.

The previous studies of Reyes et al. (US patents 5741490, 5686239 & 5770689) were merely concerned with cloning of immune reactive sequences. Immunoreactivity is shown in one, by neutralizing activity of anti-serum raised against some of these peptides (example 4, US patent 5741490); by protection of primates against HEV (example 6, US patent 5770689), and in yet another patent by ELISA (examples 4, 5 & 6, US patent 5686239).

But, it was not known from the above if any of the recombinant proteins form homodimers, nor was the immunoreactivity they detected by the different methods associated with any homodimer.

In fact, Reyes et al., did not observe dimeric or higher oligomeric forms, and such would have been prevented from forming in the Reyes method. This is because the Reyes method prevents dimeric and higher oligomeric forms of the protein and causes the monomeric form.

That is to say, following the protocol used in Reyes, et al., there is no possibility for the skilled art in the art to obtain the said homodimer to be protected in the present application.

Consequently, even if there might be high homology between said peptide or fragment, analog, polymer and chimer thereof disclosed in aforementioned patents (to Reyes et al.) and the peptide of the present application, said peptide fragments,

analogs, polymers, and chimeras as generally mentioned therein will and shall not cover the unique E2 homodimer and associated HEV immunoreactivity of the present application.

Similarly, previous studies by Khudyakov et al. (1994) is concerned with linear epitopes of the HEV structural protein only, and does not teach or suggest the homodimer to be protected of the present application.

Moreover, Li et al. (US patent 6514690) is concerned with a series of recombinant peptides of the HEV structural protein. The recombinant peptides were subject to heating at 100 °C before SDS-PAGE (example 4) and western blotting (example 5). The recombinant peptides are revealed as monomers only by this method and the immunoreactivity observed is that which is associated with monomer. The investigators did not teach or suggest a recombinant protein form homodimer.

In summary, none of these investigators had analyzed any recombinant peptides by SDS-PAGE and Western blotting using samples prepared as in the present invention with E2. Hence none show peptides homodimer.

As the propensity to form homodimer is a rare property among proteins, and in the absence of direct evidence to that effect, it is not possible for those who are familiar with the field, including the investigators themselves, to predict that any of the peptides described by Reyes et al. or Li et al. are able to form homodimer.

In the light of the general structural features of protein described earlier, it is clearly understood that the immunoreactivity of the monomeric form of the recombinant peptide described by Reyes et al. and others is attributed to both linear epitopes and conformational antigenic determinants associated with the monomeric form only.

There is simply no teaching or suggestion in the art of record as to the existence of homodimer. Actually, the conventional teaching is that only the monomer (and not

homodimer) form exists. This is because the propensity to form homodimer is a rare feature among proteins and because current knowledge on this subject teaches away from the occurrence on the basis of amino acid composition, i.e., its amino acid sequence as of primary structure alone.

Although the linear epitopes may closely similar as those contained in the proteins previously described by Reyes et al. and others, since the two types of proteins share substantial amino acid sequence homology, it is important to understand that the conformational determinants associating with E2 homodimer are distinct from the conformational antigenic determinant associated with monomeric form of the proteins described by the previous workers. The cited references simply do not teach or suggest the homodimer form, and conventional thinking is contrary to the homodimer form.

Due to the above, the applicant reiterates that E2 homodimer and associated immunoreactivity of the present application are unique and have not been previously disclosed and could not be predicted from the cited prior art. Thus, independent claims 34 and 66 and dependent claims 35-37, 45, 46, 48, 49, 54-57 and 65 are patentable over the art.

CONCLUSION

It is believed that all of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider and withdraw all presently outstanding rejections. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance. Thus, prompt and favorable consideration of this amendment is respectfully requested. If the

Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (248) 641-1600.

Respectfully submitted,

Dated: 9-7-06

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Enclosures
- Attachment A – Illustration of "E2 homodimer and associated immunoreactivity"
- Sequence Listing substitute sheets 1 to 9
- Compact Disc containing a substitute computer readable form of the Sequence Listing